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EFFICACY OF HI-6 AGAINST SOMAN AND TABUN CHALLENGES IN PRIMATES AND RODENTS (U)

by

M.G. Hamilton and Paul M. Lundy

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ABSTRACT

The bis-pyridinium oxime, HI-6, in conjunction with atropine was found to offer significant protection against multiple LD_{50} challenges with the organophosphorus compounds soman and tabun. In adult rhesus macaques, the therapeutic administration of 15 mg/kg (i.v.) or 50 mg/kg (i.m.) of HI-6 protected almost all of the animals from the lethal effects of 5 × LD_{50} of either soman or tabun.

In rats, the 24 hour protective ratio against tabun and soman was 2 and 3.5, respectively, whereas in guinea pigs these values were between 4 and 6 for both agents. No evidence was obtained for acetylcholinesterase (AChE) reactivation by HI-6 after injection of tabun in rodent tissue or after soman or tabun in primate plasma. The results demonstrate the significant therapeutic benefit of HI-6 in primates, a species specific efficacy against tabun, and argue for some mechanism of action of HI-6 at least partly unrelated to AChE reactivation.

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KEY WORDS

organophosphates

primates

HI-6

soman

therapy

tabun

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1. INTRODUCTION

The bis-pyridinium oxime HI-6 ([[[4-(aminocarbonyl)pyridino] methoxy]methyl]-2[(hydroxyimino)methyl]-pyridinium dichloride) is well recognized as an extremely effective therapeutic agent for use against potent organophosphorus cholinesterase inhibitors including soman, when used in conjunction with a centrally acting cholinolytic (Oldiges and Schoene, 1970; Wolthius and Kepner, 1978; Lipp and Dola, 1980). Nonetheless, caveats have been voiced that current evidence does not support the conclusion that HI-6 would be effective as a therapy in humans. These opinions are based on three lines of evidence. First, the therapeutic efficacy of HI-6 in primates has not been unequivocally demonstrated. Lipp and Dola (1980) were able to show that the therapeutic use of HI-6 along with atropine and the prophylactic carbamate, pyridostigmine, afforded protection against soman in rhesus macaques, but this result was questioned because of (i) the inclusion of pyridostigmine albeit therapeutically; (ii) because therapy was administered i.v.; and (iii) because the dose of atropine was quite large (up to 3.0 mg/kg).

Second, although the ability of HI-6 to restore neuromuscular function in soman poisoned skeletal muscle has been shown, rodent neuromuscular preparations poisoned with soman recover more completely than similar preparations from primate species such as marmoset, rhesus and man (Wolthius *et al.*, 1981; Smith *et al.*, 1981; Smith and Wolthius, 1983). The results pointed out an *in vitro* species-dependant aspect of some of the actions of HI-6 and led to the conclusion that HI-6 may be of limited value in humans poisoned with soman.

A third line of evidence often cited as demonstrating the limited utility of HI-6 derives from efficacy studies with the compound, primarily using mice as a model, which suggested that this oxime was therapeutically inactive against tabun (Schoene and Oldiges, 1973; Maksimovic *et al.*, 1980; Clement, 1983). Further, several investigations have found HI-6 to be completely ineffective in reactivating tabun inhibited AChE (Maksimovic *et al.*, Clement, 1982; Cetovic *et al.*, 1984) again suggesting that HI-6 was of little value in tabun poisoned animals.

Other studies (Inns and Leadbeater, 1983) also reported that HI-6 was ineffective in tabun poisoned guinea pigs. However, their particular therapeutic protocol also showed that in guinea pigs, HI-6 was relatively ineffective against soman, results which differed some what from those obtained by others. Therefore, it appeared necessary to examine some of these points more carefully and to test the effectiveness of HI-6 in tabun poisoned rodents other than mice and to establish the therapeutic benefits of the oxime in tabun and soman challenged primates.

2. MATERIALS AND METHODS

2.1 Adult rhesus monkeys (*Macacca mulatta*) of either sex were used in these studies. The animals were chemically restrained with ketamine (Ketoset, 2.0 mg/kg, i.m.) and placed in a primate restraint. The cephalic vein of one arm was cannulated (Sovereign feline, 22 gauge) to facilitate the administration of drugs and the withdrawal of blood for the determination of HI-6 levels and AChE activity. Animals were allowed 2 – 2.5 hours to completely recover from the chemical restraint before the experiments began.

The organophosphorus compounds soman and tabun were administered i.m. at a dose of 5 times the LD_{50}. In the case of soman the test dose of 32.5 μg/kg was derived from the literature value for the i.m. soman LD_{50} of 6.5 μg/kg. For tabun, since (to the best of our knowledge) the i.m. LD_{50} in rhesus monkeys has not been published, the dose was determined by taking the LD_{50} i.m./s.c ratio for soman (6.5/13.5 μg/kg) and applying this to tabun (s.c. LD_{50}: 70 μg/kg). This resulted in a calculated i.m. LD_{50} for tabun of 33.7 μg/kg and a test dose of 169 μg/kg. The agent challenge was administered into the musculature of the thigh at time 0. An i.v. infusion of diazepam (Valium, Roche, 0.2 mg/kg) was initiated immediately after the organophosphate had been injected, followed at 15 seconds by the administration of HI-6 and atropine.

Four protocols were used in the protection experiments with primates against soman. The first protocol was designed to duplicate conditions used by Lipp (Lipp and Dola, unpublished observations) so the drug doses and routes were chosen accordingly. In the first protocol the therapeutic drugs were administered i.v. during the period 15 to 30 seconds after the i.m. challenge with soman (HI-6 15 mg/kg; diazepam 0.2 mg/kg; atropine 3 mg/kg). The second protocol tested whether the beneficial effects observed in the first experiment were due to the relatively large dose of atropine. Consequently, the dose of atropine was reduced to one estimated to be "man-equivalent" (0.5 mg/kg), but the three drugs were still administered intravenously. The last two protocols, incorporated intramuscular administration of HI-6 (50 mg/kg) and atropine (0.5 mg/kg) for challenges with both soman and tabun.

Blood samples were removed through the indwelling catheter at various times after the agent challenge for measurement of AChE activity and HI-6 determinations. After the last blood sample was withdrawn at four hours, the animal was returned to his home cage.

2.2 Rodent Studies

Both male rats (Spraque-Dawley) and guinea pigs (Hartley) were used to assess the efficacy of HI-6 against soman and tabun. In experiments with rats, atropine (17 mg/kg, i.p.) was administered 30 minutes before and HI-6 (125 mg/kg, i.p.) 1 minute before the s.c. injection of the AChE inhibitor. With guinea pigs, both atropine (17 mg/kg) and HI-6 (100 mg/kg) were injected i.m. 1 minute after the organophosphate challenge. Protective ratios were calculated on the basis of survivors at 24 hours.

2.3 Determination of Acetylcholinesterase Activity

AChE was determined from 10 μL aliquots of heparinized blood using the method of Siakotos *et al.* (1969) as previously described (Lundy and Shih, 1983).

2.4 Determination of HI-6 Plasma Concentrations

HI-6 plasma concentrations were measured spectrophotometrically after separation using high performance liquid chromatography (HPLC). Plasma samples (50 μL) were mixed with an equal volume of 0.04 M $HClO_4$ containing the internal standard, obidoxime. These samples were mixed briefly and then centrifuged for 5 minutes using an Eppendorf microfuge. The supernatant was transferred to a centrifugal filtration apparatus (Schleicher and Schuell) and filtered through 0.2 micron Nylon 66 membrane filters. Aliquots (5 μL) of this filtrate were used for the quantitative analysis of HI-6.

The chromatographic system comprised a SpectraPhysics model 8700 liquid chromatography pump, an (15 cm) Ultrasphere (Altex) 5 micron reverse phase C18 column, and a variable wavelength spectrophotometric detector (Beckman Model 165) equipped with a 5 μL flowcell. The sample was introduced into the mobile phase (0.1 M H_3PO_4/KH_2PO_4, pH 4.8, containing 75 mg/L sodium octylsulphonate and 5%, by volume, acetonitrile) flowing at 1.5 mL/min using a Rheodyne injector. Peaks were detected at 304 nm. Chromatographic data was captured and analysed using a computing integrator (SpectraPhysics Model 4200).

3. RESULTS

3.1 Protection in Primates

After the i.m. administration of 5 LD_{50}'s of soman to adult rhesus monkeys, the onset of signs of organophosphate poisoning began within 30 – 60 seconds. It was found that if the infusion of diazepam was delayed by as little as 15 seconds, the initial severe convulsions produced by the agent persisted for several minutes and could result in damage to the experimental subject. Therefore, to minimize the potential damage to the animal because of the requirement for restraint, the i.v. injection of diazepam began immediately after the i.m. administration of the agent.

The animals, treated with 5 × LD_{50} of soman, regardless of the route of administration of therapy, suffered apparent convulsive activity which was manifested by dorsal flexion of the head with concomittant rigidity in the limbs. This stage lasted not more than about two minutes. As mentioned above, in the absence of diazepam the convulsions were extremely severe. During this time, the animals were semi-conscious and responded to noise and usually to touch. Following this period, which was often accompanied by at least one short lasting apneic period, the animals' respiration rate progressively increased and they became more aware and responsive. Peripheral muscle fasciculations and/or tremors were evident intermittently for the duration of the experiments and the animals were weak and incapacitated for about 24 hours. All three monkeys which had received tabun and the therapeutic regimen briefly lost consciousness in the initial one to three minute time frame. Otherwise, the course of events was similar to that seen in monkeys which had received soman.

Table 1 shows the effect of various regimens of HI-6, in combination with atropine sulphate and diazepam, on the outcome of a challenge with 5 LD_{50}'s of soman or tabun. The value of including HI-6 in the therapeutic mixture is evidenced by the fact that all animals survive without any further significant supportive therapy for at least 4 hours, and a substantial majority (82%) appear to make a full recovery. The death of one of the soman treated animals five days after poisoning was entirely unexpected as the animal was eating and drinking in an apparently normal manner. The results also illustrate that of those animals that were poisoned with soman and died, two of the three had received therapy intravenously. This along with the survival success of animals receiving therapy i.m. suggests that drug absorption from an i.m. site is not the limiting step for the success of therapy incorporating HI-6. Support for this conclusion is found in Figure 1 which shows that by 10 minutes the plasma concentration of the oxime equals or surpasses that of intravenously administered drug, and that therapeutic concentrations are likely achieved within 5 minutes.

3.2 Rodent Studies

The ability of HI-6 to protect primates from both soman and tabun challenges prompted a re-examination of efficacy in rodents. The results of these experiments are listed in Table 2. In both guinea pigs and rats, HI-6, in conjunction with atropine, afforded considerable benefit to animals poisoned with multiple LD_{50}'s of the

organophosphates. In male rats, the twenty-four hour protective ratios following a single injection of HI-6 ranged from 2 to 3 (tabun) to 3.5 (soman). However, with multiple injections of the oxime, it was possible to obtain a dramatic increase in the protective ratios to between 8 and 9 for soman and 6.6 for tabun (Lundy and Hand, submitted for publication). In guinea pigs, single i.m. therapeutic administration of HI-6 and atropine yielded protective ratios of 4 to 5 for both soman and tabun, very similar to the results described here for rhesus monkeys.

The lack of correlation between protection from lethality and AChE reactivation is shown in Table 3. In no case did HI-6 cause a significant increase in enzyme activity from tabun poisoned animals, when compared to animals treated with atropine alone. Similarly, Figure 2 demonstrates that regardless of the route of adminstration, plasma AChE activity in monkeys poisoned with 5 × LD_{50} of soman fall extremely rapidly such that by 15 minutes, AChE activity is not significantly different from zero. There is no evidence of any reactivation of enzyme activity over the four hour course of the experiment. These results are quite similar to those obtained in tissue taken from tabun poisoned rodents (Table 3).

4. DISCUSSION

The most striking aspect of the results is that in a therapeutic situation, administration of HI-6 and atropine afforded significant protection to primates and rodents against large doses of not only soman but also tabun. Several studies in mice have indicated that HI-6 may be of very limited use in tabun poisoning because it failed to afford significant protection in this species against tabun challenges and because it failed to reactivate tabun inhibited enzyme (Schoene and Oldiges, 1973; Maksimovic *et al.*, 1980; Clement, 1982; Cetovic *et al.*, 1984). Thus, based primarily on classical interpretation of the enzyme reactivating mechanism underlying the protective effects of oximes together with the demonstrated lack of protective activity of HI-6 in mice, it has become accepted that HI-6 would be devoid of therapeutic activity against tabun (Briggs and Simons, 1984; Ligtenstein, 1983).

Our results do not support this conclusion. In primates, guinea pigs and, under certain conditions, in rats, HI-6 afforded significant protection against tabun. This protection occurs, however, in the absence of any evidence of AChE regeneration which

supports earlier data (*loc. cit.*) concerning the inability of HI-6 to reactivate tabun inhibited enzyme.

A major aim of these experiments was to test the effectiveness of HI-6 in primates and to determine whether previous results detailing the beneficial effect of this oxime in monkeys (Lipp and Dola, 1980) might have been obtained as the result of the use of a relatively large dose of atropine and/or the presence in the therapeutic regime of pyridostigmine, a very useful compound when given before challenge (Dirnhuber *et al.*, 1979; Inns and Leadbeater, 1983). The results presented in Table 1 demonstrate that therapeutic success after large doses of soman depends neither on route of administration nor on the dose of atropine. In addition, because none of the protocols included the carbamate pyridostigmine, the experiments were entirely "therapeutic" in nature and had no "prophylactic" component.

The data also indicate that absorption of therapeutically active amounts of HI-6 from i.m. sites is relatively rapid. Within 10 minutes, HI-6 plasma concentrations following i.m. injection of 50 mg/kg to the monkeys approximate those of an i.v. injection. Within 3 minutes, significant amounts (12 μg/mL) are present in the primate plasma, similar to data reported for the absorption and elimination of HI-6 from an intramuscular site in humans (Kusic *et al.*, 1985). The peak plasma levels that were achieved in man occur at approximately the same time (30 min) as the peak levels reported here but the half-life of the oxime in humans not exposed to organophosphate or other drug treatments was about 3 times that found in the present work with monkeys (80 min *vs* 26 min) (Kusic *et al.*, 1985). The present results indicate that the effective therapeutic concentrations reached in monkeys given HI-6 are similar to levels reached in man following 200 – 500 mg of HI-6. This suggests, therefore that similarly effective therapeutic concentrations of HI-6 can be rapidly attained in man without untoward side effects (Kusic *et al.*, 1985) even if we assume that the dose used here in monkeys is the smallest effective dose, which is unlikely to be the case.

Regardless of the route of HI-6 administration, plasma AChE activity fell very rapidly after administration of either organophosphorus compound and approached zero within 30 min. AChE activity in the monkey blood showed no signs of recovery over the four hour duration of the experiment after administration of either agent, but this does not rule out the possibility of enzyme reactivation at some more critical sites.

However, in the rodents, no increase in AChE activity in blood, brain or diaphragm could be measured in animals which had received HI-6 and tabun. These results concerning enzyme reactivation are very similar to and support those previously reported for tabun inhibited enzyme (Maksimovic *et al.*, 1980; Clement, 1983; Cetovic *et al.*, 1984).

In summary, this bis-pyridinium oxime has been shown to possess significant therapeutic benefit in primates and rodents not only against soman but also against tabun. The lack of demonstrated evidence in these studies of AChE regeneration in either case, and particularly with tabun, argues strongly for some alternate mechanism for at least some of the actions of HI-6 (Lundy and Tremblay, 1979; Su *et al.*, 1983; Edery, 1983; Cetovic *et al.*, 1984) but does not detract from the fact that this oxime appears to be an effective and rational therapeutic strategy against all of the "nerve" agents.

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Table 1

EFFECT OF VARIOUS THERAPEUTIC REGIMES CONTAINING HI-6 ON SOMAN OR TABUN POISONING IN PRIMATES‡

ORGANOPHOSPHATE	HI-6 (mg/kg)	ATROPINE (mg/kg)	N	4 HR	24 HR	5 DAYS	14 DAYS
SOMAN	15 i.v.	3.0 i.v.	2	2	1	1	1
5 × LD_{50} (32.5 μg/kg; i.m.)	15 i.v.	0.5 i.v.	2	2	2	2∇	1
	50 i.m. + 50 i.m. at 60 min	0.5 i.m.	2	2●	2	2	2
	50 i.m.	0.5 i.m.	2	2	2	1	1
TOTALS (%)				100	87.5	75	62.5
TABUN* 5 × LD_{50} (169 μg/kg; i.m.)	50 i.m.	0.5 i.m.	3	3	3	3	3
TOTALS (%)				100	100	100	100

‡ All animals received diazepam (0.2 mg/kg, i.v.) immediately after organophosphate.

* Dose estimated as described in Methods.

● One animal was briefly respirated by hand twice between 5 to 15 min post soman.

∇ One animal died unexpectedly after 5 days.

Table 2

TWENTY-FOUR HOUR PROTECTIVE RATIOS IN RATS AND GUINEA PIGS AFTER SOMAN OR TABUN

SPECIES	TREATMENT	PROTECTIVE RATIO*	
		SOMAN	TABUN
Rat‡	Atropine + HI-6	3.5	2 to 3
Guinea Pig•	Atropine + HI-6	4 to 5	5

* Protective Ratios (LD_{50} treated/ LD_{50} untreated) were calculated on the basis of survivors at 24 hrs.

‡ The rats received 17 mg/kg atropine 30 minutes before and 125 mg/kg HI-6 1 minute before s.c. administration of agent.

• Guinea pigs received 17 mg/kg atropine and 100 mg/kg HI-6 (i.m.) 1 minute after the s.c. injection of agent.

Table 3

TISSUE AChE ACTIVITY AFTER TABUN IN RATS AND GUINEA PIGS

TREATMENT	TIME OF SACRIFICE	ACETYLCHOLINESTERASE ACTIVITY ± S.E.M. (nmoles ACh hydolysed/mg wet weight/min)			
		BRAIN		DIAPHRAGM	
		RAT	GUINEA PIG	RAT	GUINEA PIG
Control	+ 30 minutes	6.08 ± 0.48	6.28 ± 0.36	0.73 ± 0.06	0.54 ± 0.02
Tabun* + Atropine•	at death	0.51 ± 0.09	0.26 ± 0.06	0.15 ± 0.12	not detectable
Tabun + Atropine + HI-6‡	+ 30 minutes	0.17 ± 0.07	0.56 ± 0.06	0.20 ± 0.13	0.01 ± 0.006
	+ 120 minutes	0.32 ± 0.11	0.43 ± 0.11	0.35 ± 0.22	0.002 ± 0.002
	+ 240 minutes	0.38 ± 0.13	n.d.	0.05 ± 0.01	n.d.

* Tabun was administered s.c. at time 0 in at a dose of 4 × LD_{50} (rats = 920 μg/kg; guinea pig = 376 μg/kg).

• Atropine sulphate (17 mg/kg) given i.p. 15 min before (rats) or i.m. 1 min after (guinea pigs) tabun.

‡ 125 mg/kg HI-6 was administered i.p. to rats immediately before tabun and 100 mg/kg i.m. to guinea pigs 1 min after tabun.

n.d. Not determined.

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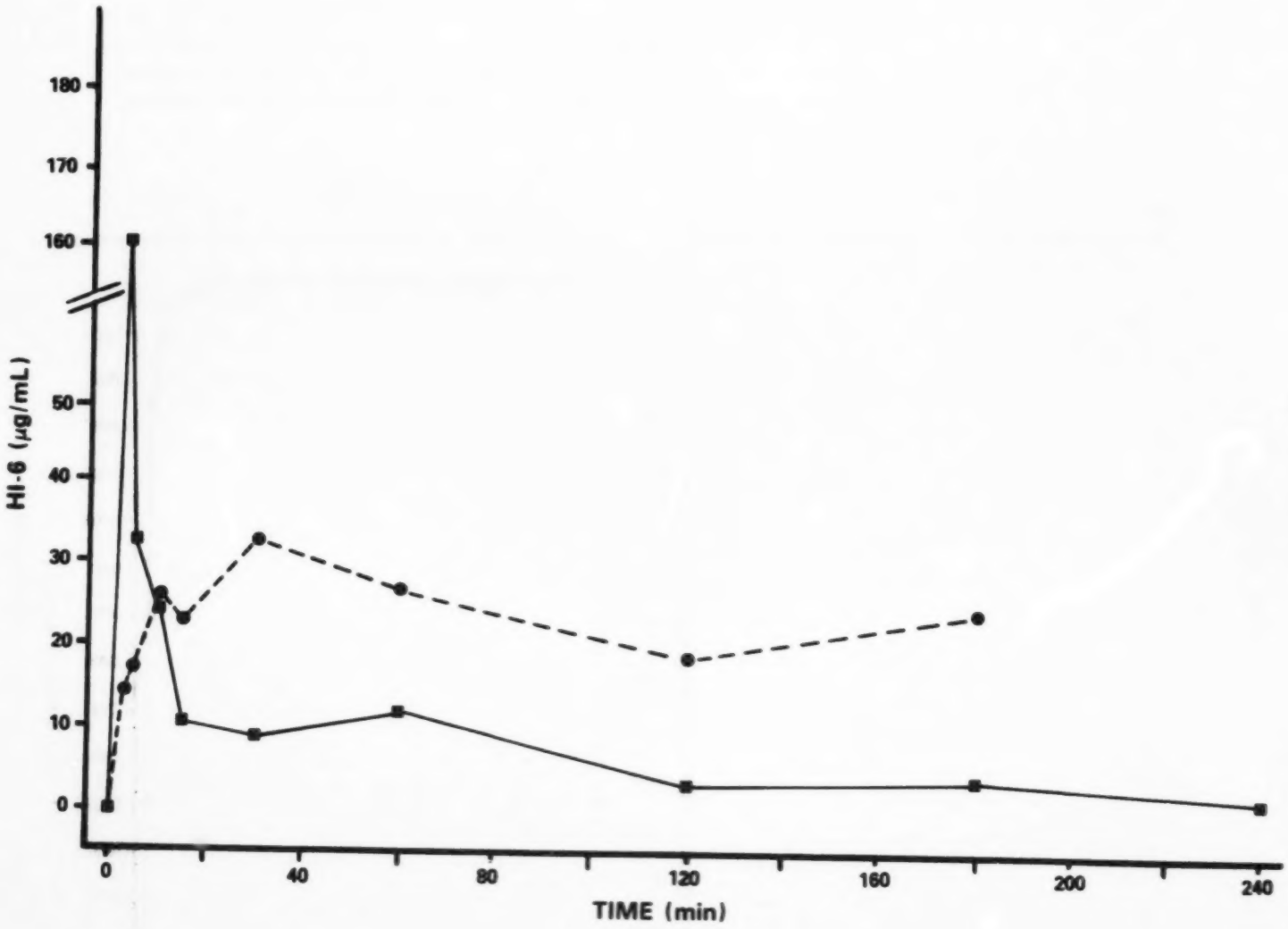

Figure 1

PLASMA CONCENTRATIONS OF HI-6 FOLLOWING I.V. ADMINISTRATION OF 15 mg/kg (■—■) OR I.M. ADMINISTRATION OF 50 mg/kg (●—●). PLASMA HALF-LIVES WERE DETERMINED TO BE 17 min (I.V.) AND 26 min (I.M.)

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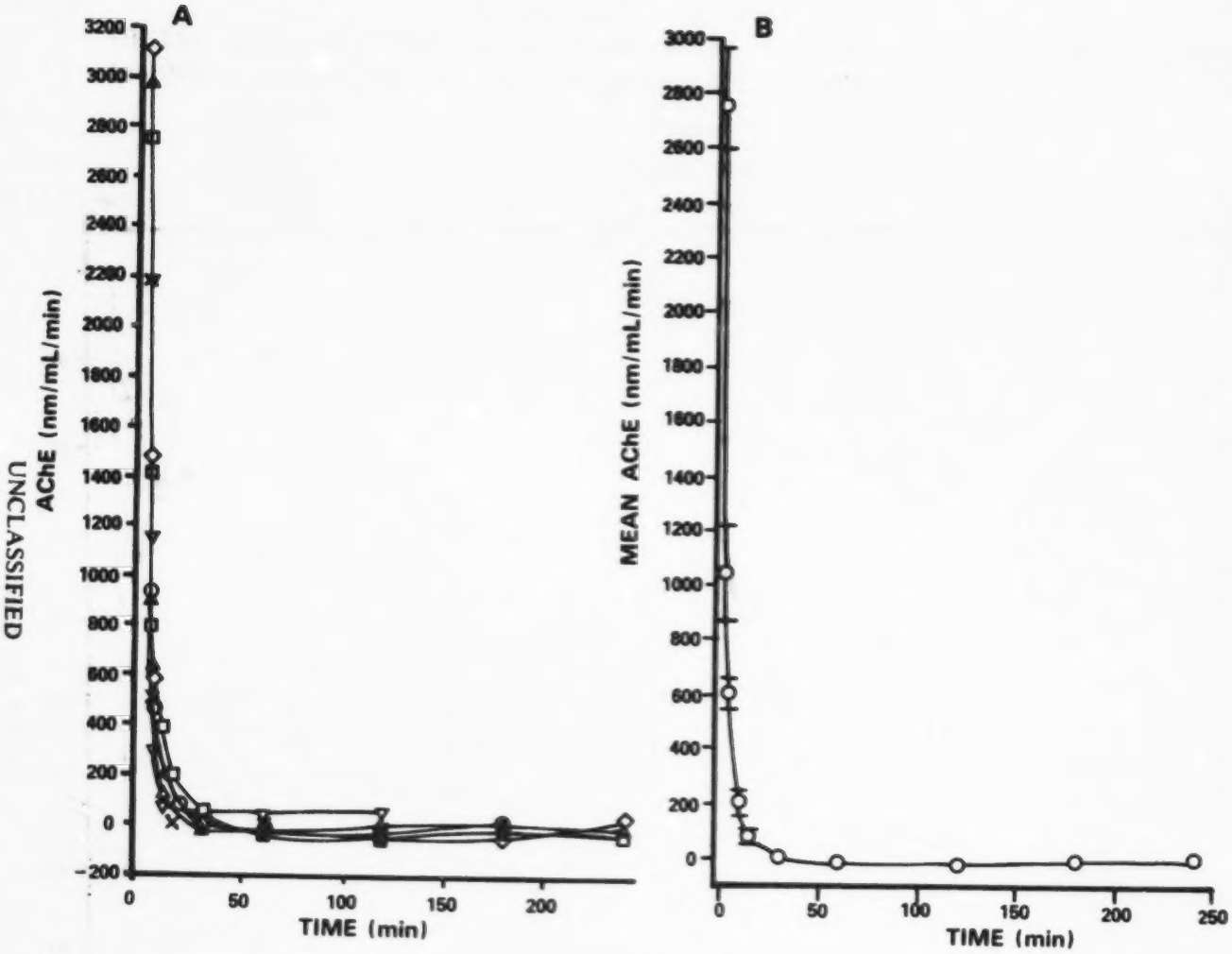

Figure 2

BLOOD ACETYLCHOLINESTERASE VALUES IN RHESUS MONKEYS AFTER 5 × LD_{50} OF SOMAN. PANEL A SHOWS VALUES FROM 3 ANIMALS THAT RECEIVED THERAPY I.V. AND 3 THAT RECEIVED THERAPY I.M. PANEL B SHOWS THE MEAN ± S.D. FOR THE 6 ANIMALS. REGARDLESS OF ROUTE OF THERAPY ADMINISTRATION, PLASMA AChE ACTIVITY FELL RAPIDLY TOWARDS 0 AND SHOWED NO EVIDENCE OF ANY REACTIVATION.

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13 ABSTRACT

The bis-pyridinium oxime, HI-6, in conjunction with atropine was found to offer significant protection against multiple LD_{50} challenges with the organophosphorus compounds soman and tabun. In adult rhesus macaques, the therapeutic administration of 15 mg/kg (i.v.) or 50 mg/kg (i.m.) of HI-6 protected almost all of the animals from the lethal effects of 5 x LD_{50} of either soman or tabun.

In rats, the 24 hour protective ratio against tabun and soman was 2 and 3.5, respectively, whereas in guinea pigs these values were between 4 and 6 for both agents. No evidence was obtained for acethlcholinesterase (AchE) reactivation by HI-6 after injection of tabun in rodent tissue or after soman or tabun in primate plasma. The results demonstrate the significant therapeutic benefit of HI-6 in primates, a species specific efficacy against tabun, and argue for some mechanism of action of HI-6 at least partly unrelated to AchE reactivation.

KEY WORDS

organophosphates
HI-6
therapy
primates
soman
tabun

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